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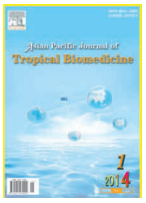

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Bovine salmonellosis in Northeast of Iran: Frequency, genetic fingerprinting and antimicrobial resistance patterns of *Salmonella* spp.

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PEER REVIEW

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Comments

This is a well-done study on the epidemiology of *Salmonella* infection in dairy farms. The importance of the article lies on the high prevalence of *Salmonella* Typhimurium as the major isolated *Salmonella* spp in the studied farms. The resistance of the isolates to several antimicrobial agents is remarkable of the public health viewpoint.

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ABSTRACT

Objective: To evaluate serovar and antimicrobial resistance patterns of *Salmonella* spp isolated from healthy, diseased and necropsied cows and calves in this observational study.

Methods: Nineteen isolates recovered from feces and tissues of salmonellosis-affected animals of two commercial farms in north-east of Iran. In second part of the study, the two farms were sampled 4 times with an interval of 2 month. The samples included calves' feces, adult cows' feces, feeds, water, milk filters, and milk fed to calves. Five *Salmonella* were isolated from 332 fecal samples collected from calves and peri-parturient cows. No *Salmonella* was recovered from water, feed, milk filers and milk fed to calves.

Results: *Salmonella* Typhimurium was the most frequently isolate among all sero-groups. S. Dublin was only accounted for 8% (two out of 24) of isolates. Isolated *Salmonella* strains were used for the ERIC PCR DNA fingerprinting assay. Our results grouped *Salmonella* isolates into 3 clusters, suggesting that specific genotypes were responsible for each sero-group of *Salmonella*. The results also revealed diversity among *Salmonella* isolates in cluster III (sero-group B). Eighteen out of 19 *Salmonella* spp. were resistant to oxytetracycline. Five isolates out of 19 showed more than one drug resistance. Multi-drug resistance was seen only among *Salmonella* Typhimurium isolates. Enrofloxacin was the most susceptible antibiotic against all isolates in this study.

Conclusion: The emergence of multiple antibiotic-resistant strains of *Salmonella* Typhimurium should be of great concern to the public. No correlation between ERIC fingerprinting and resistance patterns of *Salmonella* isolates was found, which indicates resistance to antimicrobial agents was not related to specific genetic background.

KEYWORDS
Dairy cattle, *Salmonella* Typhimurium, Antibiotic resistance, ERIC PCR

1. Introduction

Salmonellosis is a common disease of livestock animals; manifestations include diarrhea, dehydration, abortion, depressed mentation, pneumonia, septic arthritis, meningitis, gangrene of distal extremities, and sudden death. The effects of infection can range from subclinical to endotoxemia and death. *Salmonella* outbreaks can be detrimental to dairy producers due to increased mortality and treatment costs in clinically infected cows[1].

Salmonellosis is one of the most frequently reported bacterial foodborne diseases and is a major economic and public health issue worldwide. In the United States, *Salmonella* serotypes cause an estimated 1.4 million cases of foodborne disease[2] and 400 deaths annually[3]. European data show that *Salmonella* is the second most predominant

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bacterial pathogen, causing around 132 000 human cases in 2008[4]. Ninety-five percent of human cases estimated to be foodborne origin. Humans can be infected with *Salmonella* from animal sources by many routs. More cases of bovine-associated salmonellosis in humans might result from direct contact with cattle, and ingestion of foods of bovine origin (milk and uncooked beef meat)[5]. Antimicrobial resistance in *Salmonella* inhibits the ability of physicians and veterinarians to treat severe infections, and results in increases in health care costs and mortality in human patients[6].

The present study designed to provide an on-farm view of the prevalence of *Salmonella* spp. among healthy, diseased and necropsied cows and calves, to determine the serotypic diversity of *Salmonella* isolates, and to monitor antimicrobial drug susceptibility of isolates in two large scale dairy farms of north-east of Iran.

2. Materials and methods

2.1. On-farm study

2.1.1. Study population

Two commercial dairy farms belonged to the same company with the same management in north-east of Iran in the province of Khorasan were sampled. The farms were chosen due to previous history of salmonellosis. Both herds milked more than 800 cows. The farms were close herds and did not receive animals from other herds. Cows were housed in open shed with sand and straw for bedding. Non-lactating cows and heifers were housed in separate pens. The calves were housed in individual cement hutches. Calves were typically fed un-pasteurized milk. Non-salable milk (*i.e.*, milk not allowed for human consumption) was fed to calves. Milk was fed with buckets.

2.1.2. Sampling procedure

Farms sampled 4 times with an interval of 2 month. Types and number of samples collected at each visit were summarized in Table 1. The samples included calves' feces, adult cows' feces, feeds, water, milk filters, and milk fed to calves. Fresh fecal samples were collected directly from healthy animals by rectal grab. Milk filters were washed with peptone water into a sterile bottle. All samples were collected between July 5, 2009 and March 8, 2010.

2.2. Diagnostic study on clinical cases

From June 2007 to March 2010, samples of diseased and necropsied animals from farms A and B were referred to the Diagnostic Laboratory of Center of Excellence in Ruminant Abortion and Neonatal Mortality, Ferdowsi University of Mashhad. Six *Salmonella* were isolated from feces of diarrheic and 13 isolates from tissues of necropsied animals. These isolates were used for sero-typing and antimicrobial susceptibility testing. No other pathogen was isolated.

The isolation methods of *Salmonella* spp. are based on Cobbold *et al.* (2006)[7]. Samples transported to the laboratory besides ice bags. Five grams of each fecal sample added to 45 mL of tetrathionate broth and enriched for 48 h at 37 °C. Liquid samples such as milk and water (60-80 mL) were combined with equal volume of double concentration selenite-F broth and enriched for 24 h at 37 °C. Solid samples such as feeds at the amount of 25 g was added to 225 mL of buffered peptone water, mixed thoroughly, and pre-enriched for 24 h before being added to tetrathionate broth and re-enriched for 24 h at 37 °C.

All enrichments were streaked for isolation on McConkey agar plates and incubated at 37 °C overnight. Lactose negative colonies from each plate were confirmed as *Salmonella* on the basis of growth pattern (alkaline/acid+H2S and urea negative) on triple-sugar iron and urea agar slants. Isolates were stored at nutrient broth with 15% glycerol at -20 °C for future reference.

2.3. Serogrouping

Salmonella isolates were grouped by use of a commercial slide agglutination method (Kooshafar Biotechnology Research Institute, Karaj, Iran).

Table 1
Types and numbers of samples collected on visits to 2 dairy farms during farm study.

		Feces						Raw milk fed to calves	Milk filters	Water	Feeds
		Calves<2 weeks	Calves 2-4 weeks	Calves 1-4 month	Calves 4-6 month	Close-up cows and heifers	Fresh cows and heifers				
Farm A(Z)	1st Sampling	8	5	10	6	10	5	1	1	1	1
	2nd Sampling	5	8	5	5	10	14	1	1	1	2
	3rd Sampling	5	5	5	5	10	9	1	1	1	1
	4th Sampling	5	5	5	5	10	10	1	1	1	1
Farm B(G)	1st Sampling	5	5	5	5	10	10	1	1	1	2
	2nd Sampling	5	5	7	5	10	11	1	1	1	1
	3rd Sampling	5	5	5	5	10	10	1	1	1	2
	4th Sampling	5	5	4	5	10	10	1	1	1	1
Total		43	43	46	41	80	79	8	8	8	11